

cLTP and cLTD protocols

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Updated date: Jul 22, 2020



An abbreviated version of this protocol was published in eLIFE in May 2015

Synaptic activity regulates AMPA receptor trafficking through different recycling pathways

DOI: 10.7554/eLife.06878

Detailed protocol

cLTP and cLTD protocols (adapted from protocol provided by Michael Ehlers' lab)

Buffers:

Imaging buffer: NaCl (150mM), KCl (5mM), CaCl₂ (2mM), MgCl₂ (2mM), HEPES (10mM), D-Glucose (30mM), pH=7.34-7.36

Mg²⁺-free buffer: Imaging buffer above, but omit MgCl₂, and add 0.02mM bicuculline and 0.001mM strychnine. Bicuculline and strychnine 1000x stocks can be stored at -20 °C. pH=7.34-7.36

Mg²⁺ buffer: Mg²⁺-free buffer supplemented with 2mM MgCl₂.

Glycine buffer: Mg²⁺-free buffer plus 0.2mM glycine. Make fresh each time (glycine should be made fresh from powder).

cLTP:

1. Wash the neurons 1x with imaging buffer to get rid of the culture medium. Then 2x with Mg²⁺-free buffer.
2. Change Mg²⁺-free buffer to glycine buffer and incubate the neurons at 37°C for 15 minutes.
3. Wash the neurons 1x with imaging buffer then incubate the cells in Mg²⁺ buffer (2mM MgCl₂) for 30 minutes (37°C).

cLTD:

NMDA buffer: Mg²⁺-free buffer plus 20μM NMDA (NMDA stock can be stored at -20 °C).

1. Wash neurons 3x with Mg²⁺-free buffer.
2. Incubate neurons in NMDA buffer at 37°C for 5 minutes.
3. Wash the neurons 1x with imaging buffer then incubate the cells in Mg²⁺ buffer (2mM MgCl₂) for 1h (37°C).

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Jeyifous, O. (2020). cLTP and cLTD protocols. Bio-protocol Preprint. bio-protocol.org/prep410.
2. Zheng, N., Jeyifous, O., Munro, C., Montgomery, J. M. and Green, W. N. (2015). Synaptic activity regulates AMPA receptor trafficking through different recycling pathways. eLIFE. DOI: [10.7554/eLife.06878](https://doi.org/10.7554/eLife.06878)

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